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BIOLOGICAL BULLETIN

FORM-REGULATION IN CERIANTHUS, V.

The Rôle of Water-Pressure in Regeneration: Further Experiments.

C. M. CHILD.

Reduction of Water-Pressure by Means of Artificial Openings and its Effect on Regeneration.

Early in the course of my experiments the apparent relation between internal pressure and the rapidity of tentacle regeneration was noted. The facts obtained incidentally led me to believe that it would be possible to influence the course of regeneration by altering the internal pressure. The results of experiments have amply justified this belief, though I hope at some future time to be able to apply to the problem new methods which have suggested themselves, and thus to eliminate some of the complicating factors.

The problem was attacked experimentally by various methods which may be grouped under two heads: one group of methods consisted in the frequent reopening or spreading of the margins of an artifical opening in the body-wall, the other in the attempt to make an artificial opening in the body-wall of such a form that complete closure and consequent distension of the piece with water would be impossible or greatly delayed. The chief difference in the two sets of methods is that with the first, the pieces are reopened at stated periods, every alternate day, every day, or oftener, while with the second they remain undisturbed after section. The results obtained by these two methods differ little and afford strong evidence in favor of the view that water-pressure is an important factor in regeneration in *Cerianthus*.

Pieces Reopened at Intervals.

In experiments of this kind the results are more or less modified by certain complicating factors which it is difficult to eliminate completely. Some discussion of these is necessary before proceeding to an account of the experiments.

The region of the piece selected for opening was in most cases the aboral end simply in order that the region subjected to the irritation connected with repeated manipulation might be as far removed as possible from the regenerating region. As a matter of fact it was found that the manipulation itself, even though frequently repeated and including extensive section or tearing of the tissues, exerted little or no influence on the course of regeneration in other regions of the piece.

The description in the third paper of this series ('04*a*) of the process of inrolling of the body-wall after section in consequence of the elasticity of certain of its layers explains the necessity for repeated opening of a piece when continued communication between the enteron and the exterior is desired. In practice it was found that within a half hour after section the opening was usually reduced to mere slits and crevices by the approximation of its margins. Moreover, the ectodermal slime, which plays an important part in the provisional closure of wounds, as was described in the third paper ('04*a*), aids in plugging the small spaces between the approximated margins. In consequence of these two factors, the inrolling of the body-wall about a cut and the presence of slime which plugs the crevices, the attempt to keep the enteron in constant communication with the exterior through an artificial opening meets with great difficulties. The attempt was made to avoid these complicating conditions by introducing a glass tube into the body through the opening, but even when the tube was provided with a lip to hold it in place the pieces succeeded in creeping away from it, and when it was secured by a ligature the body separated at the ligature if this was drawn tightly, or gradually drew itself out if the ligature was loosely tied. All attempts to keep the end open by the introduction of some foreign body failed.

The only other possibility in pieces of such a form that closure is possible is the repeated opening and spreading apart of the inrolled margins to permit escape of the water from the enteron. From what has been said above regarding the rapidity of infolding of the margins and the closure by means of slime, it is evident that this reopening must be frequent if the establishment of water-pressure in the enteron is to be entirely prevented. In the course of my experiments of this kind it was found practically impossible to prevent closure by approximation of the margins and plugging of the opening with slime between the times of opening. Pieces in which the mouth had regenerated, thus permitting rapid entrance of water, were often found more or less distended with water after being left over night. Frequently even a shorter time than this was sufficient to permit provisional closure and the establishment of more or less pressure in the enteron. It is clear therefore that unless such pieces are reopened at very short intervals — one to two hours — the establishment of some degree of water-pressure in the enteron cannot be prevented in many cases. It was impossible for me to reopen the pieces every hour or two during several days or weeks, consequently in no case was it possible to eliminate entirely water-pressure in the enteron.

As a matter of fact, however, the water-pressure in a piece in which one end is closed only by approximation of the margins and plugging of the crevices with slime is in most cases much below the pressure in normal animals, as could be readily determined by the resistance of the body-wall, the degree of distension, etc. It follows from this fact that pieces whose aboral ends are repeatedly opened and not allowed to close definitively by the formation of new tissue will be subjected to much less pressure than those allowed to close in the usual manner. It is possible then to reduce the enteric pressure if not to eliminate it entirely, and as my experiments will show, the reduction in pressure has a marked effect. In my experiments, the pieces were opened at intervals of from two days to half a day.

Except where statement to the contrary is made all experiments described here were performed on *Cerianthus solitarius*. Each series retains the number given it in my notes and the date of each observation is given. Comparison of the control pieces will show wide differences in the rapidity of regeneration in different series, due of course to the temperature of the water at the time of experiment (cf. Child, '03*b*).

Series 48.

November 21, '02. — Ten large species of *C. solitarius* were cut transversely a short distance aboral to the œsophagus (Fig. 1), the portions aboral to the cut being used for experiment. In five of these pieces the body was split longitudinally for half its length from the aboral end and oblique incisions were made at various points on the longitudinal cut surfaces (see dotted lines in Fig. 1). The object of this procedure was to produce great irregularity in the cut surfaces at the aboral end in order that the inrolled edges might not fit closely together but leave numerous spaces between them, thus permitting communication between the enteron and the exterior. These five pieces may be designated as the experimental pieces or the experiment.

The remaining five pieces were not injured aborally; they were placed in the aquarium under the same conditions as the first five and remained undisturbed, serving as a control.

From the beginning of the experiment to December 8 the experimental pieces were reopened on alternate days, *i. e.*, the slime was cleaned from the aboral ends and with the aid of needles and forceps the end was spread widely open, care being taken in each case to ascertain that the communication between the enteron and the exterior was unobstructed. From December 9 to December 17 they were reopened daily. The terms employed in describing the various stages are explained by reference to the first paper of the series (Child '03*a*).

November 28. — 7 days after section. Experimental pieces; no tentacles present; tentacular ridge not distinct; oral end not expanded.

Controls: one piece distended with tentacular ridge appearing; other pieces partly filled; tentacular ridge not distinct.

December 2. — 11 days after section. Experimental pieces: A few minute tentacle-buds on two pieces; two others with tentacular ridge just appearing. All four of these pieces have succeeded in closing aborally more or less perfectly and becoming more or less distended in the intervals between the reopening of the aboral end; the fifth piece remained widely open aborally and shows no trace of tentacles.

Controls: All with distinct tentacular ridge and some tentacle-buds just appearing.

December 8. — 17 days after section. Experimental pieces: Since these differ somewhat from this stage on, each piece is specially designated by a letter which it retains throughout the remainder of the experiment. In pieces *a* and *b* the longitudinal cuts extend only about half the length of the piece, in *c* about two thirds, while in *d* and *e* they extend more than three fourths the length.

(*a*) In this piece the longitudinal cuts are less deep than in the others and the end closes more perfectly being less irregular. At each examination before reopening the piece was more or less distended with water. Marginal tentacles 1 mm.; oral end partially expanded.

(*b*) This piece also succeeded in closing the aboral end more or less perfectly by approximation of parts between the times of reopening. Marginal tentacles 0.5–1 mm.; oral end partially expanded, a small area of new tissue visible at center.

(*c*) Has been only very slightly distended at any time: aboral end has remained partly open most of the time except for slime. The tentacular ridge bears only minute buds; the oral end is not expanded sufficiently to show the new tissue in the center.

(*d*) Has been widely open aborally since December 2: aboral end torn in reopening and margins rolled inward in such manner that a wide communication between enteron and exterior remains. The oral regeneration of this piece has not advanced visibly since December 2; the oral end is still unexpanded and new tissue not visible at the center; a tentacular ridge is barely visible but tentacle buds are wholly absent.

(*e*) Has remained most widely open aborally of all throughout the experiment: oral end wholly unexpanded; no tentacular ridge and no trace of tentacles visible.

Controls: All five pieces with oral ends expanded and showing a distinct area of new tissue at center; tentacles 1–1.5 mm.

December 12. — 21 days after section. Experimental pieces. Since December 8 have been reopened daily:

(*a* and *b*) Oral ends slightly expanded, diameter less than that of body; marginal tentacles 1.5–2 mm., slightly longer in *a* than in *b*; blunt and less slender than normal tentacles (Fig. 2).

(*c*) Oral end not expanded; very minute tentacle buds on tentacular ridge.

(*c*) Oral end not expanded; no tentacular ridge or tentacles visible.

Controls. Marginal tentacles 2–3 mm. (Fig. 3, one of controls).

December 17. — 26 days after section. Experimental pieces. Reopened daily since December 12, but *a* and *b* have been slightly distended at times. During the course of regeneration the mouth has formed in the usual manner, though more slowly

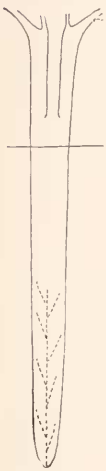

Fig. 1.

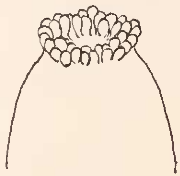

Fig. 2.

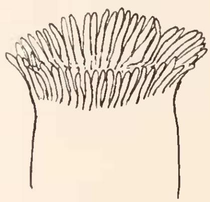

Fig. 3.

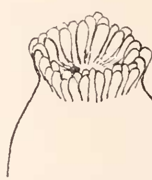

Fig. 4.

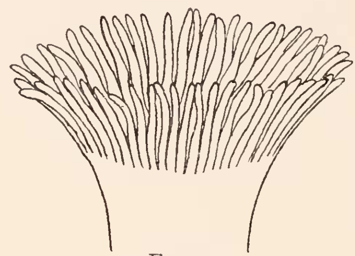

Fig. 5.

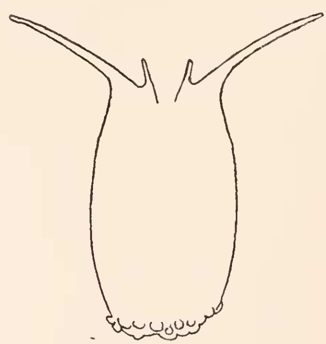

Fig. 6.

than in the controls, consequently water is now taken in much more rapidly than in the earlier stages.

(*a*) Oral end slightly expanded; marginal tentacles 3–4 mm., blunt and less slender than normal; no labial tentacles visible (Fig. 4).

(*b*) Oral end slightly expanded; marginal tentacles 2–3 mm., blunt and less slender than normal; no labial tentacles.

(*c*) Oral end not expanded; marginal tentacles 1–2 mm., blunt; no labial tentacles.

(*d*) Oral end not expanded; marginal tentacles 0.5 mm., no labial tentacles.

(*e*) No tentacles visible.

In all experimental pieces the diameter of the disc, where such is distinguishable, is, and has been throughout, less than the diameter of the body (Fig. 4), resembling in this respect the earliest stages of typical regeneration.

Controls. In four pieces marginal tentacles 6–8 mm. (Fig. 5), in fifth about 5 mm.; all with labial tentacles about 0.5 mm.

In all of the controls the diameter of the disk is greater than that of the body, the whole being somewhat trumpet-shaped like the normal animal.

From this time on the experimental pieces were left undisturbed in order to determine whether they still had the power to close and complete the retarded regeneration. The controls were discarded.

December 28. — (*a*) Aboral end closed by new tissue; body distended with water; marginal tentacles 5–6 mm., slightly more blunt than normal tentacles but much less blunt than at previous examination; labial tentacles 0.5 mm.

(*b*) Aboral end closed by new tissue; body distended; marginal tentacles 7–8 mm., still somewhat more blunt than normal; labial tentacles 1 mm.

(*c*) Aboral end closed by new tissue; body distended; marginal tentacles 6–7 mm., slightly more blunt than normal; labial tentacles 0.5 mm.

In all three pieces (Fig. 6) the diameter of the disc is still somewhat less than that of the body, but is relatively greater than on December 17, *i. e.*, the oral end is expanding. The aboral ends bear slight knobs and irregular masses of old tissue, which represent small shreds and strips of the old body-wall which had rolled into rounded masses during the experiment and are now undergoing absorption.

(*d*) New tissue at aboral end does not close end completely as yet; body is only slightly distended; marginal tentacles 1–1.5 mm., blunt; labial tentacles minute buds.

(*e*) Widely open aborally, about as before in appearance.

January 11, 1903. — (*a*) Body distended in normal manner but still shorter and more sac-like than normal; marginal tentacles 12–15 mm.; labial tentacles 4–5 mm.

(*b*) Distended, form like that of *a*; marginal tentacles 10–12 mm.; labial tentacles about 3 mm.

(*c*) Distended, similar to *a* and *b* in form; marginal tentacles 7–8 mm.; labial tentacles 1–2 mm.

(*d*) Now completely closed and distended, similar to others in form: marginal tentacles 6–7 mm.; labial tentacles 1–1.5 mm.

(*e*) Has never closed aborally; oral end not expanded; minute marginal tentacle-buds visible; no labial tentacles.

Series 12.

September 12, 1902. — Six specimens were cut transversely just below the œsophageal region (Fig. 7). Three to be used as controls and three as experimental pieces.

September 15. — 3 days after section: In all six pieces the oral end was closed with delicate new tissue and all were partly filled with water. Three of the pieces were now split longitudinally from the aboral end for half their length (experimental pieces), the other three remaining undisturbed (controls). The experimental pieces contracted so strongly during this operation that the new tissue closing the oral end was ruptured, but the tentacle-forming region was not injured. The cut aboral ends of the experimental pieces are to be opened morning and evening of each day to prevent distension as far as possible.

September 19. — 7 days after first operation. Experimental pieces. Have not been distended at any time, although some accumulation of water has occurred in the intervals between the operations; the oral end of one not expanded and the new tissue merely fills crevices; two pieces with minute marginal tentacle-buds visible; in the third formation of tentacle-buds beginning at a few points on the tentacular ridge.

Controls: All well-filled with water and disc expanded; the new tissue at oral end forms a distinct central area in consequence of distension; one piece with marginal tentacles 2 mm.; two pieces with tentacles 1 mm.

September 22. — 10 days after first operation. Experimental pieces. Form of disc in all cases as before; two pieces with marginal tentacles 1 mm.; third piece with tentacle-buds just visible.

Controls: All well-filled, with expanded discs; marginal tentacles in all cases 3–5 mm.; labial tentacles appearing.

The experimental pieces were now allowed to close and become distended.

September 24. — 12 days after operation. Experimental pieces. All distended, oral ends expanded but less so than in controls; all with marginal tentacles 2–3 mm.; one piece with labial tentacles just visible.

Controls: Marginal tentacles 5–7 mm.; labial tentacles 1 mm.

These two series are sufficient to serve as illustrations of the methods. In other series in which the same methods were employed the results were essentially the same, so that it is unnecessary to give the data for others in detail.

In a few series the new tissue at the oral end was punctured with a lancet as soon as it formed and then this end kept open by daily insertion of the lancet between the inrolled margins. Special care was taken in these operations not to injure the tentacle-forming region which lies a short distance from the cut margin (cf. the first paper of this series '03*a*), but to destroy only the delicate membrane and so allow communication between the enteron and exterior. It is unnecessary to give the details of this series since the results obtained are essentially similar to those of other series. In every case the diameter of the oral end remained less than that of the body, the appearance of the tentacles was delayed and the regeneration retarded as long as the pieces were kept open. Tentacle-buds when formed on open pieces were blunt and rounded, and after a time became whitish in color and opaque. When allowed to close the oral end began to spread in the typical manner and the tentacles grew as rapidly as in the controls.

Delayed Closure in Pieces of Certain Forms.

In my third paper (Child, '04*a*) some of the varieties of inrolling after section were described and it was shown that in pieces of certain forms, especially those which were split longitudinally, inrolling often occured in such manner as to delay or even prevent union of the cut surfaces and thus the closure of the piece. Such pieces live for months in many cases, undergoing gradual closure from some region where approximation of cut surfaces renders possible the formation of new tissue between them. Sometimes the end or ends close readily but closure along the side is delayed and in cases of spiral inrolling closure of the end is possible only on the innermost part of the spiral and closure of the sides, not at all. These pieces present an almost infinite variety, but in all their forms afford important evidence as to some of the formative factors in *Cerianthus*. Without recognition of the method of growth of new tissue between cut surfaces, and the relation between tension due to internal water-pressure or other causes and growth of new tissue almost every one of these pieces might be described in detail — for scarcely any two are alike — as an "abnormality." If, however, we attempt to study the special conditions and the reactions to them in each case we find that the differences and "abnormalities" are not difficult to interpret. A number of pieces in which closure was prevented or delayed by the manner of inrolling are described below. These are selected from a much larger number which show various differences in detail from those described. I have endeavored, however, to select pieces which should show as far as possible the principal "abnormalities." The account will serve both as evidence upon the question of water-pressure and regeneration and as a description of the course of regeneration in the respective pieces. In most cases these experiments were performed without controls, so that it is impossible to determine exactly how much the first appearance of the tentacles is delayed; it will be sufficiently evident, however, that it is delayed. In the figures illustrating the inrolling of these pieces the lines employed for shading were so drawn that they also indicate in a general way the longitudinal striping of the body and thus show the direction in which the various parts are rolled inward.

Series 11.

September 12, 1902. — Three specimens were cut transversely a short distance aboral to the œsophagus (Fig. 7) and then split longitudinally on one side (Fig. 8). After section they were allowed to remain undisturbed.

September 19.—Seven days after section. Examination showed that none had closed, the cut margins rolling in on all sides having rendered closure impossible. Figs. 9 and 10 show two of the pieces as they appeared at this time. In the case of Fig. 9 the longitudinal cut separated both sides of the body at the aboral end and one of the lobes thus formed overlaps the other in the figure. The cut margins at the oral end are inrolled and united by new tissue much as in a cylindrical piece, but aboral to this region the two margins separated by the longitudinal cut have rolled in independently leaving a large open space between them. On the oral end are a few minute tentacle-buds — far below the normal number.

In Fig. 10 the inrolling has been somewhat different: on the right side of the figure it is much the same as in Fig. 9 but on the left, instead of rolling longitudinally the oral and aboral parts of the wall have rolled more or less transversely and in opposite directions. At the oral end only a part of the cut surface is exposed, the remainder being rolled inward behind the visible portion in the figure. The exposed portion of the oral end, corresponding to the longitudinally inrolled part of the body-wall is closed by thin new tissue where opposing cut surfaces are approximated in the inrolling, and bears a few minute tentacle-buds.

The third piece differs in detail from the other two as regards inrolling, but resembles them in that a few minute tentacle-buds are present and the enteron is widely open to the exterior.

In the first case (Fig. 9) the transverse section of each inrolled portion would show the form of a spiral with the longitudinal cut surface forming its innermost end: the same is true of the part on the right of Fig. 10, though here the inrolling is less complete. In these portions the contact between different parts of the body-wall is sufficiently close to permit a slight accumulation of water in the inner regions of the spiral and therefore a slight distension. The pressure in these parts never approaches that in the normal animal; as soon as it reaches a certain point escape of the water occurs between the approximated surfaces. Considering the tentacles, we find that their appearance is delayed. Cylindrical pieces closing in the typical manner regenerate tentacles of the size shown in Figs. 9 and 10 in from four to five days at this season of the year. These pieces required seven days and the circle of tentacles is by no means complete. Only those portions of the oral end which are exposed and not completely collapsed bear tentacles.

These pieces were examined at intervals of a few days during three months.

November 1. — 49 days after section. The pieces were much reduced in size owing to absence of food. During this time the tentacles had shown no farther growth, but had decreased in size so that they were scarcely visible. A part of one of the pieces

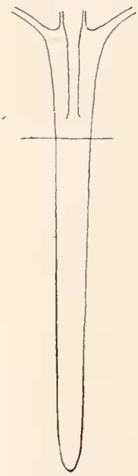

Fig. 7.

Fig. 8.

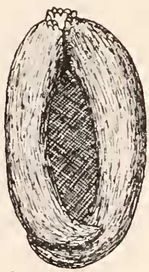

Fig. 9.

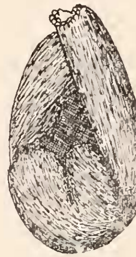

Fig. 10.

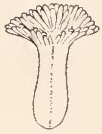

Fig. 11.

had constricted off, a not uncommon occurrence in cases of long continued collapse or severe injury.

November 10. — 58 days after section. It was found that the piece represented in Fig. 9 was finally closing. During the experiment the new tissue had been gradually extending from the ends of the longitudinal cut, slowly drawing the cut edges together, the last portion to close being near the middle.

The tentacles of this piece are now increasing in size again, being 0.5–1 mm. in length.

The other pieces have not closed, though in each some growth of new tissue along the cut surface has occurred. In these pieces the inrolling occurred in such manner that the elasticity of the new tissue was insufficient to approximate the cut edges.

November 20. — 68 days after section. Tentacles of the closed piece 1–2 mm.; scarcely visible on other pieces, which are still open. Fig. 11 shows the closed piece as it appears at this time. The figure is drawn to same scale as Fig. 9, thus indicating marked decrease in size. The longest tentacles on the specimen are opposite the longitudinal cut; on each side of the cut the tentacles are very short; all tentacles are rather blunt. The new tissue uniting the edges of the cut is indicated by stippling.

This piece lived until December 28, and one of the others until December 12. During this time they become still farther reduced in size, but the second piece never closed.

Certain of the special features of this series require brief comment. It will be noted that even after closure the tentacles of the closed piece did not grow to any great extent, although some growth did occur. This failure to grow is probably due to the exhausted condition in consequence of absence of food. This may effect the power of growth, *i. e.*, the reactive capacity of the tissues, or it may cause a decrease in the water-pressure in one or both of two ways; first, it is quite probable that soluble products of katabolism accumulate in the enteron much less rapidly than earlier, owing to the decreased metabolism; if this is the case diffusion of water into the enteron and consequently distension of the enteron will be much less rapid: on the other hand there can be little doubt that the beat of the cilia is less powerful in these nearly exhausted specimens than in normal individuals. This being the case, it is easy to see that the inward current through the siphonoglyphe is not capable of maintaining as high internal pressure as in a normal animal: moreover, the local circulatory currents into the tentacles must also be less powerful. It is not improbable that all three of these factors play a part in the result; the osmotic factor is more important before the mouth is formed, decreased ciliary power afterward.

Series 17.

September 14, 1902. — Eight specimens were cut in the same manner as in Series 11 (Fig. 8) and allowed to remain undisturbed after section.

September 22. — 6 days after section. None of the pieces closed and none showed any tentacle-buds. On ordinary cylindrical pieces regenerating tentacles appear in about four to five days at this time of year and are usually 1 mm. long on the sixth day.

September 28. — 12 days after section. Four pieces possessed minute tentacle-buds on at least a part of the oral end. The pieces were variously rolled, those with tentacles at least in part longitudinally, the other four in part transversely. All were more or less widely open in the middle region. Two of the pieces at this stage are shown in Figs. 12 and 13. In Fig. 12 the portion on the right has rolled longitudinally in a spiral and at the oral ends the opposing margins of the spirally rolled portion are united by new tissue and bear minute tentacles. The remaining portion of the oral end is rolled aborally behind the tentacle-bearing portion, much as in Fig. 10.

In Fig. 13 the inrolling is almost wholly longitudinal and at the oral end new tissue has united the whole circumference. The longitudinal cut margins are rolled inward, but not united, though closely approximated. The circle of tentacle-buds is almost complete.

October 5. — 18 days after section. Six of the pieces are rolled transversely in a spiral, having changed somewhat in form since the last examination. In one of these in which the transverse inrolling began at the aboral end a few tentacles 2 mm. in length are present; in the other pieces there was no change as regards tentacles. The elongation of tentacles in this one piece was apparently due to the accumulation of some water in the outer turn of the spiral, *i. e.*, the more oral part of the piece, the margins of this turn being applied to the next inner turn closely enough to permit the establishment of some degree of water-pressure.

A few days later all eight of the pieces were dead, probably on account of accidental insufficiency in the water-supply.

Series 28.

September 26. — Nine specimens were cut transversely aboral to the œsophagus, then the aboral end was removed and the pieces were slit longitudinally on one side. Thus these pieces resemble those of Series 11 and 17 (Fig. 8) except that a few millimeters at the aboral end have been removed. The object in the removal of the extreme aboral end in pieces was to avoid if possible the transverse inrolling from the aboral end which was so frequent in Series 11 and 17 (Figs. 10 and 12) and which interferes greatly with closure. In this series then the object was to

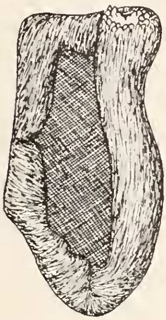

Fig. 12.

Fig. 13.

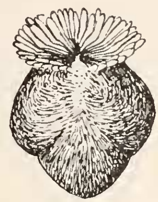

Fig. 14.

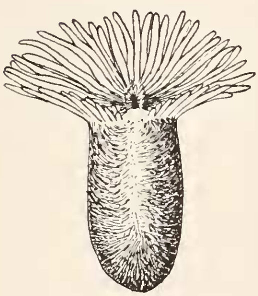

Fig. 15.

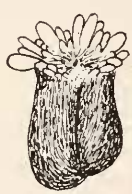

Fig. 16.

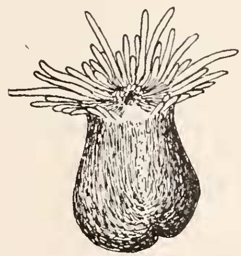

Fig. 17.

delay closure for a time but not to make it impossible. The experiment was continued for about four months and during that time all of the pieces closed and acquired essentially the typical form of the species.

October 3. — 7 days after section. None closed; variously rolled; only two with any traces of tentacle buds.

October 10. — 14 days after section. Six pieces with tentacles; three pieces still without any traces of tentacles.

From this point on it will be convenient to give the history of each piece up to its closure separately. The pieces are numbered 1–9 in the order of their closure.

In each case the first date given for a piece is approximately the time when the elongation of the tentacles from the minute bud-like stage began. In every case then the regeneration of the tentacles has been retarded up to, or nearly to, this first date. In a few cases closure occurred a few days before the first date given so that the tentacles had attained a length of several millimeters by the time of examination. In almost every case the ends closed first and from each end the new tissue gradually extended along the cut towards the middle.

No. 1. October 10: Closed, oral end expanded, marginal tentacles 5–7 mm. on half of disc opposite the longitudinal cut, near the cut 2–3 mm.; labial tentacles just appearing on side opposite cut, not present on cut side.

October 17: Marginal tentacles 10–12 mm. on one side, 5–6 mm. on the other; labial tentacles 1–2 on uncut side, just appearing on cut side.

October 24: Marginal tentacles 12–15 mm., almost equal on all parts of disc except a short space without tentacles in region of cut; labial tentacles 3–4 mm., nearly equal on all parts.

No. 2. October 24: Closed; just becoming well distended; marginal tentacles 3 mm.

November 1: Marginal tentacles 6–8 mm.; labial tentacles just appearing, etc.

No. 3. October 10: Not closed by new tissue, but cut edges rolled in such manner that slight distension is present; marginal tentacles 3 mm., blunt and sac-like.

October 24: Still about as before.

November 1: Closed and distended. One marginal tentacle 6–7 mm., others 3–5 mm.; labial tentacles just appearing.

November 10: All marginal tentacles 7–8 mm., etc.

No. 4. November 1: Not fully closed by new tissue, but slightly distended and with sac-like tentacles 2–3 mm.

November 20: Closed and distended. Marginal tentacles 4–5 mm.; labial tentacles just appearing, etc.

No. 5. November 20: Just closed and becoming distended. Marginal tentacles 2–3 mm. on uncut side, reduced or absent on cut side (Fig. 14).

December 2: Marginal tentacles 6–7 mm. on uncut side, 3–0 mm. on cut side; labial tentacles present on uncut side, absent on cut side (Fig. 15).

December 12: Marginal tentacles 10 mm. on uncut side, 5–0 mm. on cut side, etc.

No. 6. December 2: Apparently not yet fully closed by new tissue, but slightly distended; marginal tentacles 2–3 mm., blunt and sac-like.

December 12: Apparently closed, distended; one marginal tentacle 5 mm., others 2–3 mm.; labial tentacles just appearing.

December 24: Marginal tentacles irregular in length, 3–6 mm., labial tentacles 1–2 mm., etc.

No. 7. December 6: Closure by new tissue not complete, but slightly distended, some marginal tentacles 2 mm., sac-like, others mere buds (Fig. 16).

December 12: Closed and distended. Marginal tentacles very irregular in length, 1–6 mm., absent near region of cut; a few labial tentacles just appearing (Fig. 17).

December 24: Marginal tentacles 3–7 mm., absent near cut; labial tentacles 1–2 mm. on uncut side, a few absent on cut side.

No. 8. December 24: Closed and distended. Marginal tentacles 4–5 mm., quite regular; labial tentacles 1 mm., a few absent near region of cut.

January 21, 1903: Little change since December 24.

No. 9. January 21: Apparently closed, but distension slight, probably because of low temperature, slime, and continued starvation; marginal tentacles 1–3 mm., mostly 3; labial tentacles 1 mm.

In this series the close relation between the internal water-pressure and growth of the tentacles is evident. Although the marginal tentacles appeared as minute buds before marked distension occurred, they did not advance beyond this condition in any case until the water-pressure increased — in some cases a period of more than three months. As soon as distension took place the tentacles continued their regeneration. This series constitutes a demonstration of the rôle of water-pressure as a factor in the regeneration of the tentacles, but does not throw any additional light upon the problem of their origin and localization.

Delay or prevention of closure was accomplished by various other methods and since the results in all cases agree with those of the series already described an account of all experiments along this line would be merely a multiplication of detail to little purpose. A summary of the principal other methods employed and the results obtained will be sufficient.

Series 31.

September 28. — Seven specimens were cut transversely aboral to the æsophagus as in Fig. 8 and then slit longitudinally down one side for three fourths of their length, differing from Fig. 8 in that the longitudinal cut did not extend all the way to the aboral end. These pieces closed much more readily than those of Series 11, 17 and 28. In this series as in Series 28 some of the pieces closed and became distended much sooner than others. The earliest distension of the body and elongation of tentacles occurred 14 days after section. Thirty-eight days after section all of the pieces were completely closed and with marginal tentacles more than 5 mm. in length. The delay in closure was not as great in most cases as in Series 28, but the results as regards delay in tentacle-regeneration agree in all respects with those of that series.

In two series — 18 and 20 — 8 and 6 pieces respectively were separated by a transverse cut aboral to the œsophagus as in Fig. 7, and then the oral half of the piece was split into two parts by a longitudinal cut through both sides of the body. In consequence of this cut the oral part of the piece was doubled, the aboral remained single. In a few cases specimens with double oral ends and separate sets of tentacles resulted, but the more common results were the separation from the piece of the divided parts at its aboral end or the union of the two to form an individual essentially normal.

It will be evident at once, however, that the inrolling after such an operation must have been rather complex. Closure of the openings by new tissue was usually slow whatever the particular result, and delay in the regeneration of the tentacles occured here in the same manner as in the series alaeady described. The "double-headed" specimens will be described in another connection.

A similar splitting of the aboral end was accomplished in Series 19 and 38 — 6 and 4 pieces respectively. Three of these pieces produced specimens with double aboral end, but in the remainder the two parts united more or less completely. In these series delay in union of the cut surfaces also occurred, and whenever the presence of openings prevented distension the tentacle-regeneration was delayed, proceeding only when distension took place.

In several series the attempt was made to prevent union of cut surfaces at the aboral end by making the cut surface very irregular in form, splitting it into strips, making oblique cuts in it, removing portions, etc., the object being to bring about independent inrolling of different parts and thus prevent the complete closure of the end by new tissue. These experiments were not very effective, however. A greater or less delay in closure and a consequent delay in tentacle-regeneration was brought about, but in nearly every case closure took place within two or three weeks and after that regeneration proceeded in the usual manner.

General Considerations.

It is, I think, sufficiently demonstrated by the forgoing experiments that there exists a close relation between internal water-pressure and tentacle-regeneration. In order, however, to meet possible objections and to render more clear certain points a brief discussion of the results is necessary.

The objection may be made that the operations are severe enough to produce some pathological effect upon the pieces which prevents or retards their regeneration. Any objection of this nature is abundantly refuted by the data of the experiments themselves, from which it is clear that just as soon as the enteron is capable of holding water under some degree of pressure the course of regeneration becomes typical, and that even where the regeneration has been delayed for one or two months it is as complete when it does occur as if it had taken place immediately after section, although the resulting animal is smaller. Of course where the pieces are kept without food for periods of three or four months or more they become greatly reduced in size and their capacity for regeneration is diminished; nevertheless, even such pieces as these show very clearly the result of internal pressure. A good example is the piece of Series 11 shown in Fig. 11 and the last pieces of Series 28.

In the preliminary survey in the preceding paper (Child, '04*b*) it was suggested that not only does the rapidity and amount of tentacle-regeneration depend to a greater or less extent upon the internal water-pressure, but the position of the regenerating marginal tentacles may be determined by the local pressure upon the inrolled oral end of the piece caused by the circulatory current which moves orally in the peripheral part of each mesenterial chamber and thus continually forces water into the blind sac at the oral end produced by the inrolling of the body wall and the mesenteries bounding each chamber. According to this view the tissue reacts to this local change in pressure by local growth and the position of the new marginal tentacle is determined. The first visible changes leading to regeneration consist in the reduction in thickness of the body-wall in consequence of the disappearance in large part or completely of the longitudinal muscle-layer. The local area of thin tissue being thus formed, it is effected both by the local pressure due to the circulatory current and by the general internal pressure. Both of these factors subject the area to tension and, as in other areas of thin "new" tissue (see the section on "The Closure and Distension of Pieces" in the preceding paper, Child, '04*b*), growth is the result.

A few tentative suggestions based upon the preceding data are added here in order to indicate the bearing of certain features of the experiments upon this hypothesis. But first of all the fact should be emphasized that water-pressure constitutes in any case only one factor in a complex process; it is impossible to doubt its influence in view of the experimental data, but recognition of the rôle played by water-pressure does not constitute a complete solution of the problem of form-regulation in *Cerianthus*.

It should be borne in mind that complete elimination of local water pressure due to currents has not been accomplished in any of the experiments. The methods employed have succeeded in bringing about a more or less complete reduction of the general distension of the body by water but the local circulatory currents due to cilia continue of course wherever water is present in the intermesenterial chambers. These, however, must be considerably reduced in volume and force for several reasons: first, in the contracted condition of the open pieces the surface area of the intermesenterial chambers is much less than in distended specimens and the mesenteries are often so closely approximated that little water can enter the chambers; second, the entodermal surface is more or less folded and wrinkled in the contracted condition and so the number of cilia effective in producing currents is reduced; third, it is possible, though this is mere surmise, that the cilia beat less rapidly when the body is less distended, since the impeded circulation of water may reduce their supply of oxygen sufficiently to bring about such an effect. There can be little doubt then that the circulatory currents are at any rate reduced in volume and force in the open pieces. We find in such pieces a delay in the regeneration of the tentacles in comparison with pieces which are allowed to close and become distended in the usual manner. The fact that tentacles do finally appear in open pieces is due, according to this view, to the impossibility of eliminating completely by the methods employed the circulatory currents in the intermesenterial chambers. I hope in future experiments to be able to eliminate these currents more completely than has been possible with the methods employed thus far, and so to determine whether tentacle-regeneration can be completely inhibited by this means.

In open pieces the tentacles do not regenerate completely, but remain of small size as long as the pieces are open. As soon as closure and distension occur, however, they increase in length. The difference in the force of the circulatory currents in open and closed pieces may account for this difference. The assumption is justifiable that a certain degree of pressure causes a certain amount of growth, not indefinite growth, since the tissues as they grow apparently become less sensitive to the stimulus, or react to it in some unexplained manner so that growth gradually ceases. The so-called functional adaptation in bone, in the circulatory system, and in various other tissues indicates that this assumption is correct. Admitting this we may apply it to the case in hand and conclude that the failure of regenerating tentacles to grow beyond a certain small size in open pieces is due to the slight degree of local pressure exerted by the circulatory currents.

In many open pieces the regenerating tentacles appear only on a part of the oral circumference or appear later on some parts than on others or grow more rapidly or to larger size on some parts than on others. Figs. 9–17 afford examples of all these conditions. In pieces which close and regenerate in the typical manner no such differences occur. All these irregularities in open pieces may be regarded as due to differences in the force and volume of the circulatory currents in different intermesenterial chambers. In parts which are much contracted or tightly rolled little or no water can enter, and consequently the circulatory currents are slight or absent. The fact that in cases of partial tentacle-regeneration the parts on which tentacles appear are more or less distended, while other parts are collapsed, confirms the conclusion. In general, cases of this kind are similar to those described in the preceding paper (Child, '04*b*) under the head of "Local Inhibition of Tentacle-Regeneration." In these cases, as in those described in that paper, it will be noted that the parts most strongly contracted or folded are those which fail to produce tentacles. In Fig. 9 both halves of the piece become slightly distended and tentacles appear on both except near the longitudinal cut where the edges are strongly inrolled. In Fig. 10 only the right side of the piece is capable of retaining water under pressure and only here do tentacles appear. The same is true of Fig. 12, while Fig 13 resembles Fig. 9 in that both sides become more or less distended. Figs. 14 and 15 show an interesting case in which the longitudinal cut surfaces have become greatly shortened and the mesenteries bent at an acute angle: these mesenteries are so closely approximated to each other that little or no water can enter between them and we find the tentacles corresponding to these chambers either minute and delayed in regeneration or absent. In Figs. 16 and 17 a case is shown in which closure was long delayed and the folds and wrinkles about the oral end were present for so a long a time that when the piece finally did close and become distended there were marked irregularities in the size of different intermesenterial chambers and consequently marked differences in rate of growth and size of tentacles. The larger tentacles belong to those chambers which happened to be more or less widely open during the period of collapse. Some of the tentacles did not appear at all until complete closure and distension had occurred. In all cases after closure and distension the irregularities were gradually reduced until an approximation to the typical condition was attained. It seems scarcely necessary to multiply details further. My observations and experiments have led me to believe that in every case of this kind it can be shown that absence of tentacles or delay in their appearance is correlated with contraction, folding, etc., which may prevent the occurrence of circulatory currents in the intermesenterial chambers or reduce their force and volume.

The form of the marginal tentacles in open pieces is usually markedly different from that of typical tentacles, the former being blunt and rounded in form, often almost sac-like, instead of slender and tapering. A comparison of Figs. 2 and 4 with Figs. 3 and 5 affords an illustration of these differences; Fig. 16 represents an extreme case of this kind. In all such cases the blunt tentacles increase in length and soon acquire an almost or quite normal form after closure occurs. This peculiar form of the tentacles in open pieces may be regarded as due indirectly to the reduced size of the margin of the disc in these more or less collapsed pieces (cf. Figs. 2 and 4 with 3 and 5). Since the margin of the disc is reduced the opening into the tentacle from the intermesenterial chamber is smaller than in distended pieces, consequently the movement of water through it is impeded. Thus the local pressure exerted by the circulatory currents must be to a large extent eliminated within the tentacles, or at any rate insufficient to cause continued increase in their length. The general internal pressure, however, must be the same in the tentacular cavity as in the mesenterial chambers and it is possible that this has some effect upon the thin new tissue of the tentacles in causing enlargement in all directions.

It is possible also that the form of the tentacle may be affected to a greater or less extent by the rate of growth. In the open pieces the rate of growth is always slower than in closed pieces in consequence of the lower pressure in the former. Material which is employed in closed pieces in producing increase in length of the tentacle, may, in the absence of the usual powerful stimuli to longitudinal growth, be used in regions where stimuli are less powerful and so in consequence of the general pressure in all directions bring about an atypical increase in the transverse diameter of the tentacle. According to this view the stimulus to longitudinal growth in closed pieces is so much stronger than the others that growth occurs almost wholly in this direction; this stimulus being reduced others become more effective. The reactions of various forms, both growth-reactions and motor-reactions, in response to stimuli might be cited in support of this view, for it is well known that in many cases the predominant stimulus determines the reaction.

Attention has already been called to the fact that in the open pieces with delayed tentacle-regeneration the tentacles usually acquire an arrangement in two or three rows much earlier than in the closed pieces with typical regeneration. I believe that this difference is due to the smaller circumference of the disc and the rounded, more or less sac-like form of the tentacles in open pieces. It is clear that the smaller the disc the fewer the tentacles which can be borne in a single row upon its margin; and when the tentacles themselves are less slender on pieces with the smaller discs than on the typical pieces, it follows that the crowding of some of them out of the single row must occur all the earlier. Figs. 2 and 4, as compared with Figs. 3 and 5, are good examples of this difference. In the closed, typical pieces, where the disc begins to expand as soon as regeneration begins, the tentacles are found mostly in a single row until a comparatively late stage of regeneration, when they are gradually forced into two or three rows. In the open pieces, on the other hand, the blunt, sac-like tentacles often form two more or less complete rows a short time after their first appearance. This difference between open and closed pieces, which can scarcely be explained on any other basis, affords very strong evidence in favor of the view that the arrangement of tentacles in the normal animal in about three rows is the result of mutual pressure. It is possible that slight differences in the circulatory currents, themselves due to differences in lengths of mesenteries or other structural or functional features may determine the order in which the tentacles are crowded out of the single row, and thus more or less exactly the definitive mutual positions of the tentacles. Thus far, however, I have been unable to discover any such differences.

The small size of the disc in open pieces as compared with closed pieces has been mentioned frequently in the above discussion. Comparison of Figs. 2 and 4 with Figs. 3 and 5 shows the marked difference. In closed pieces the disk soon acquires greater diameter than any other part of the body while in open pieces, its diameter is usually considerably less, and never much greater than that of the more or less completely collapsed body. This difference, like the other noted, can scarcely be due to anything but differences in internal pressure. In the regenerating piece growth of new tissue at the oral end occurs at the cut surfaces and on the tentacular ridge, and the distance between these two regions of growth being slight, the intervening tissue soon becomes involved in the changes. These changes consist, as was noted in my first paper (Child, '03*a*), of reduction in thickness of the body-wall and the production of new tissue. The result is that the whole oral end of the piece becomes covered with thin new tissue, which reacts much more readily to pressure than the old thick tissue of the body-wall aboral to it. The internal pressure, although no greater here than elsewhere in the body, must cause more rapid increase in size here since the tissue is much less capable of resisting tension. If we take into consideration the circulatory currents which strike the body-wall around the whole margin of the disc we have an additional factor causing growth of the disc in the marginal portions. On the oral side of this region is the area of thin tissue composing the more central portions of the disc, on the aboral side the thick body-wall. If the former responds more readily to stimuli than the latter, which appears to be the case, it is possible to understand why the disc spreads laterally and acquires a greater diameter than the other parts of the body. At first glance it might appear that if the growth of the disc were due to the pressure of water it should grow only in the oral direction, but I think that the presence of the mesenteries and the different conditions of the new tissues on the two sides of the region of maximum pressure is sufficient to account for the fact that a spreading of the disc as well as growth in the oral direction occurs. The disc is never flat, but always somewhat funnel-shaped, indicating that the margins are the regions of most rapid growth. It is possible that actual invagination of the ectoderm at the center of the disc to form the œsophagus does not occur, but that the surrounding parts are pushed orally and peripherally by the internal pressure, leaving this portion behind, as it were, since it is firmly attached to the mesenterial margins. Goette ('98) states that the development of the œsophagus in the young *Cerianthus* occurs in this manner, though he does not express himself regarding causes.

Although the above suggestions have been made in more or less positive form, since that was more brief and convenient, I desire to state once more that I am well aware of the hypothettical character of many of them. That the general internal water-pressure does play an important part in the regeneration and reëstablishment of the characteristic form in *Cerianthus* I believe the experiments already described have demonstrated. As regards the details, however, and especially as regards the effect of local pressure due to circulatory currents extended experiment is necessary before final conclusions are possible. These suggestions I have made in the hope that they may aid both in making clear the interpretation which seems to me possible and in pointing the way for future experimentation. In later papers I shall bring forward additional evidence upon the problem.

Summary.

1. In pieces which are kept open or opened repeatedly at the aboral end the appearance and growth of the tentacles, the growth of the disc, and in fact all regenerative phenomena at the oral end are delayed. The marginal tentacles which appear under these conditions are short, blunt, and more or less sac-like in form. Labial tentacles rarely appear at all until closure and distension has occurred.

2. In pieces cut in such manner that closure is prevented or delayed retardation of regeneration occurs as in the cases just mentioned.

3. In all pieces in which closure is delayed by any means the process of regeneration resumes its typical course after closure and distention are permitted to occur.

4. It is believed that these experiments demonstrate the important influence of the general internal water-pressure upon regeneration in *Cerianthus*. Complete elimination of internal pressure was impossible with the methods employed, therefore complete inhibition of regeneration cannot be expected in any case.

5. The retardation in regeneration of the tentacles and expansion of the disc may perhaps be due essentially to the decreased force and volume of the intermesenterial circulatory currents in open as compared with closed pieces.

6. Irregularities, such as local absence of tentacles, differences in time of appearance and local differences in size may be due to local differences in the circulatory currents which in turn are the result of local foldings, inrollings, or contractions of the body-wall.

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